Virus-producing cell lines

The virus-producing cell line is generated by transfecting pG-MtkPL into the ψ-2 packaging cell line. Virus was prepared from the filtered culture medium of transfected ψ-2 cell lines. NIH3T3 fibroblasts were infected by G-MtkPL, and clones were isolated in a Phleomycin-containing medium.

RESULTS

To assay for I-*Sce* I endonuclease activity in mammalian cells, NIH3T3 cells containing the G-MtkPL provirus were used. The G-MtkPL provirus (Fig. 25a) contains the *tk* gene (in place of the *gag*, *pol* and *env* viral genes), for negative selection in gancyclovir-containing medium and, in the two LTRs, an I-*Sce* I recognition site and the *PhleoLacZ* fusion gene. The *PhleoLacZ* gene can be used for positive selection of transduced cells in phleomycine-containing medium.

We hypothesized that the expression of I-*Sce* I endonuclease in these cells would induce double-strand breaks (DSB) at the I-*Sce* I recognition sites that would be repaired by one of the following mechanisms (illustrated in Fig. 25): a) if the I-*Sce* I endonuclease induces a cut in only one of the two LTRs (Fig. 25-b 1 and 2), sequences that are homologous between the two LTRs could pair and recombine leading to an intra-chromosomal homologous recombination (i.e. by single strand annealing (SSA) (12B, 10B) or crossing-over); b) If the I-*Sce* I endonuclease induces a cut in each of the two LTRs, the two free ends can religate (end joining mechanism (31B) leading to an intra-chromosomal recombination (Fig. 25-b 3); or alternatively c)